ANALYSIS OF COLLAGEN STATUS IN WOMEN WITH GENUINE STRESS INCONTINENCE

Dissertation submitted to

THE TAMILNADU DR.M.G.R. MEDICAL UNIVERSITY

In partial fulfilment of the requirements
for the award of the degree of

M.CH (UROLOGY) – BRANCH – 1V

THE TAMILNADU DR.M.G.R. MEDICAL UNIVERSITY
CHENNAI

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DECLARATION

I solemnly declare that this dissertation titled **ANALYSIS OF COLLAGEN STATUS IN WOMEN WITH GENUINE STRESS INCONTINENCE** was prepared by me in the Department of Urology, Rajiv Gandhi Government General Hospital, Chennai under the guidance and supervision of Prof. R. Jeyaraman, M.S, M.Ch (Uro)., Professor & Head of the Department, Department of Urology, Rajiv Gandhi Government General Hospital, Chennai. This dissertation is submitted to The Tamil Nadu Dr. MGR Medical University, Chennai in partial fulfilment of the university requirements for the award of the degree of M.Ch Urology.

Place: Chennai **DR. K.HEMALATHA**
Date:

CERTIFICATE

This is to certify that the dissertation titled **ANALYSIS OF COLLAGEN STATUS IN WOMEN WITH GENUINE STRESS INCONTINENCE** submitted by Dr.K.HEMALATHA appearing for **M.Ch (Urology)** degree examination in August 2013 is a bonafide record of work done by her under my guidance and supervision in fulfilment of requirement of The Tamil Nadu Dr.MGR Medical University, Chennai. I forward this to The Tamil Nadu Dr. MGR Medical University, Chennai.

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INTRODUCTION

Genuine stress urinary incontinence is defined as a condition in which involuntary urine loss occurs in an intact urethra, when intra vesical pressure exceeds intra urethral pressure without a detrusor contraction during physical exertion.

Genuine stress urinary incontinence results from an anatomical defect of the support of the urethrovesical junction and proximal urethra resulting in displacement of these during stress, but the precise mechanism is poorly understood. Intact functional collagen fibres are needed for mechanical stability of the genitourinary tract, which support the bladder neck, urethra and pelvic organs.

In a study by Ulmsten et al and Versi et al showed alterations in collagen synthesis and types are casually related to genuine stress urinary incontinence. Low levels of collagen is found in vesicovaginal fascia, round ligaments, uterosacral ligaments as well as tissues supporting the urethra and bladder neck in women with genuine stress urinary incontinence than continent women.

Immunohistochemical analysis of paraurethral tissue for collagen changes suggests that significant role in the maintenance of urinary continence is due to connective tissue that consists mainly of collagen fibres.

AIM AND OBJECTIVE

The aim of this study is

- To study the collagen status in peri urethral vaginal tissues
- To determine the role of collagen in the maintenance of urinary Incontinence

REVIEW OF LITERATURE

HISTORY OF AETIOPATHOLOGY

By most patients, incontinence of urine is called as loss of bladder control. It is the unwanted loss or leak of urine. It is a symptom. It is not a disease. It is caused by a variety of conditions.

It is common in millions of women. It causes great embarrassment to them. One out of every four women has urinary incontinence. Urinary incontinence is common after the age of 30 years. Most of the women mistakenly believe that due to aging, they are having incontinence.

The effective continence mechanism depends on urethral support .Descend of bladder neck and urethra is caused by loss of structural urethral support. This loss of urethral support causes urethral hyper mobility. It is the main aetiology of stress urinary incontinence.

Normally bladder and urethra are placed in a high retro pubic position. The increased intra abdominal pressure is transmitted equally to bladder and urethra. When stress causes urethral descent, the position of the urethra is altered. The increased intra abdominal

pressure is not transmitted equally to bladder and urethra. Urinary incontinence results when bladder pressure is more than the urethra

Various theories have explained the continence mechanism. DeLancy et al (1994) stated that continence depends on the stability of hammock like musculofacial layer. It is not due to the urethral position. Normally the urethra is well supported by the arcus tendineus fascia pelvis and levator ani. This prevents urinary leak.

The bladder neck and urethra descends down with failure of the supporting mechanisms. The another theory stated by Plzak and Staskin (2002), that urinary continence depends on the integrity of urethral complex. It is formed by pubo urethral ligaments and pubo urethralis muscle of the levator ani.

Petros and Ulmstein (1992) stated the integral theory of stress urinary incontinence. The pelvic floor muscles, the bladder neck and the anterior pubococcygeus muscle work together. This combined work prevents stress urinary incontinence.

McGuire and colleagues (1980) stated the intrinsic sphincter deficiency theory. Leakage of urine is common after malfunction of the urethral sphincter.

Intrinsic sphincter deficiency is one of the cause for leakage of urine. Urethral hyper mobility is another principal cause of leakage of urine. Both the defects may be present in the same patient.

STUDIES REGARDING COLLAGEN CONTENT IN STRESS URINARY INCONTINENCE:

Skin, fat and round ligament from stress urinary incontinent females were studied by Ulmsten et al in 1987. Skin specimens found to have 40% less collagen. Round ligament tissue had 25% less collagen.

Falconer et al in 1994 studied skin biopsies from stress urinary incontinent females. He discovered skin fibroblasts produced 30%less collagen in these women. According to him, altered connective tissue metabolism was the main aetiology to this problem.

Norton et al discovered a reduced collagen type1:111 ratios in stress urinary incontinent females. Recurrent stress urinary incontinence is due to altered collagen synthesis.

Makinen et al in 1986 found decreased fibroblasts .Altered collagen fibril orientation was also noted by him. Vesico vaginal fascial specimens of stress urinary incontinent females were studied

by Rechberger et al. He found the fascia of incontinent women had less collagen.

Sayer et al in 1990 showed modification in the collagen cross link in the pubo cervical fascia. Keane et al showed reduction in collagen content. He also found reduced collagen type1:type111 ratio in stress urinary incontinent females. According to them, endopelvic fascial composition and vaginal epithelial composition resemble each other.

Hence pelvic floor dysfunction is contributed by a spectrum of connective tissue abnormalities. Increased focus on connective tissue changes will give better idea to plan for more effective management.

STRESS URINARY INCONTINENCE

According to literature, it is defined as involuntary loss of urine. It occurs through intact urethra. It occurs without detrusor contraction with increase in intra abdominal pressure.

TYPES

Urodynamically, the classification as follows,

Type-0

History of stress urinary incontinence is present. At rest, the bladder neck is closed. The proximal urethra is closed. They are present at or above the superior margin of pubic symphysis. In the presence of stress they open. But, there is no urine leak.

Type-1

At rest, there is closure of the bladder neck. The proximal urethra is also closed. At or above the inferior margin of pubic symphysis, the bladder neck is seen. The proximal urethra is also present above this level. There is descend of <2cm with stress. There is opening of the bladder neck with stress. The proximal urethra also opens at the time of stress with increase in intra abdominal pressure. Urinary leak is seen.

Type-2a

At rest, there is closure of the bladder neck. The proximal urethra is also closed. At or above the inferior margin of pubic symphysis, the bladder neck is seen. The proximal urethra is also present above this level. There is descend of >2cm with stress. There is opening of the bladder neck with stress. The proximal urethra also opens at the time of stress with increase in intra abdominal pressure. Urinary leak is seen.

Type-2b

At or above the inferior margin of pubic symphysis, the bladder neck and proximal urethra are present. During stress, the urethra further descends down. Urethra also opens. There is urine leak.

Type-3

At rest, there is opening of the bladder neck and proximal urethra. There is urinary incontinence without detrussor contraction. It is due to force of gravitation. It is also seen with increase in minimal intra abdominal pressure.

SUPPORTING MECHANISMS OF PELVIC FLOOR

Normal continence in females depend on the balance of several factors like,

- Urethral closing pressure
- The anatomical and functional length of urethra
- Ability of the pelvic floor to increase the urethral pressure at the time of stress
- The proper anatomic location of the sphincter unit

- The proximity and alignment of the urethra to the pubic symphysis

A leak proof mechanism depends on the factors,

- An intact urethra
- Coapting mucosal surface
- Reflux pelvic contraction at the time of pelvic strain.

Failure of one the components along with hyper mobile urethra will invariably cause stress urinary incontinence.

ANATOMY OF PELVIC SUPPORT

The structures which constitutes the pelvic support and sphincter support are as follows,

- Levator ani muscle
- Pelvic ligaments
- Pelvic fascia
- Urethral sphincters

FEMALE URETHRA

Compared to males, female urethra is short. It is about 4cm in length. It extends from bladder neck to the exterior world.

The wall of the urethra contains four layers,

- Inner mucosa
- Sub mucosa
- Muscles (inner longitudinal and outer circular)
- Outer adventitial layer

URETHRAL SPHINCTERS

Female urethral sphincter is made up of two sphincters. They are internal urethral sphincter and external urethral sphincter.

Internal sphincter is seen at the level of neck of the bladder. Detrusor muscle forms this sphincter. The muscle extends on the either side of the urethra. Normally, the bladder and the posterior urethra are kept empty. It is due to the natural tone of the internal urethral sphincter. Whenever there is increase in bladder pressure, the urine leak is prevented by the internal urethral sphincter.

Internal urethral sphincter is made up of smooth muscle. It is supplied by pelvic autonomic nerves. They are under involuntary control.

External urethral sphincter is seen at the level of uro genital diaphragm. It is made up of skeletal muscles. It is innervated by

pudental nerve. These sphincters are under voluntary control. With consciousness, the involuntary loss of urine can be prevented by the external sphincter.

External urethral sphincter contains two components. They are intra mural part and extra mural part. Intra mural part is also known as rhabdosphincter.

The shape of the external urethral sphincter is signet ring. When these sphincters are contracted, the walls of the urethra can be pulled. They are pulled posteriorly towards the perineal body.

External urethral sphincter is made up of slow type and twitch type fibres. Twitch type fibres may be slow twitch type or fast twitch type fibres.

The characters of slow twitch fibres are,

- Use oxidative metabolism
- Are recruited slowly
- Slowly undergo fatigue
- To maintain the sphincter tone for prolonged periods.

The characters of fast twitch fibres are,

- Use anaerobic metabolism
- Are recruited slowly
- Undergo fatigue rapidly
- To maintain continence when there is rapid increase in intra abdominal pressure.

Females have more effective external sphincter muscles than males. The external urethral sphincter is made up of the compressor urethrae, the sphincter urethrae, and urethro vaginal muscles.

Urethra and vagina are surrounded by urethro vaginal muscles. Contraction of both urethra and vagina occurs on contraction of these muscles.

The compressor urethrae muscles origin from right and left inferior pubic ramus. It surrounds the urethra. It squeezes the urethra against the vagina on contraction.

The peak urethral closing pressure in females depends on,

- The pseudo stratified columnar epithelium - They form folds on contraction to occlude lumen.

- The sub mucosa – it is rich in blood vessels and soft connective tissue. It forms urethral sealing.
- The smooth muscle of the urethra (longitudinal and circular Muscles)
- The Striated sphincter
- The pubo urethral part of levator ani

Compared to males, the female urethra is short. The proximal bladder neck mechanism is weak. Distal urethral mechanism is more prone for damage during birth injury. It is more prone for manipulation by external mechanisms.

PELVIC FLOOR MUSCULATURE

The main important pelvic floor muscle is levator ani. Its main function is to carry the weight of the pelvic contents and to prevent the stretching the ligamentous support structures by preventing abdominal pressure transmission.

Levator ani muscle comprises of three parts. They are pubococcygeus, iliococcygeus and pubo rectalis muscle. Puborectalis muscle extends from pubic bone to rectum. The shape of puborectalis muscle is u shape. Pubococcygeus muscle extends

from coccyx to pubis. Iliococcygeus muscle is horizontal in nature. It extends laterally from arcus tendineus levator ani. It covers the posterior opening of the pelvis. It acts like a shelf for pelvic organs to lie.

There is an aperture in the levator musculature. It is called as urogenital hiatus. The urethera and vagina come out to the exterior through this urogenital hiatus. The levator ani muscle is supplied by pudental nerve.

Levator ani muscle has both type 1 and type 11 fibres. Type 1 fibres are slow twitch. They are striated muscle fibres. They provide resting tone. Anterior compression of urethra and vagina against the pubic bone is by this muscle. The constant closure of hiatus depends on the constant muscle tone of type 1 fibres.

Type 11 fibres are fast twitch fibres. The main function of type 11 fibres is to urethral closure pressure maintenance. They also prevent stretching of the pelvic ligaments.

LIGAMENTS

The ligamental supports which are seen in the pelvis are,

- The cardinal ligaments

- Uterosacral ligaments
- Pubo urethral ligaments.

The functions of these ligaments are to give support to the pelvic organs.

Urethro pelvic ligament extends from tendineus arc. It extends up to urethra. The main function of urethro pelvic ligament is also to give fascial support to the urethra. Pubo urethral ligament extends from inferior side of pubic symphysis. It extends up to the mid urethra. The downward rotational descend of the urethra is prevented by this ligament.

Mid urethral complex is more important to prevent incontinence. It is formed by pubo urethral ligament and pubo urethralis muscle. Pubo urethralis muscle is a part of levator ani muscle. A sling around the mid urethra is seen. It is formed by pubo urethral ligament and pubo urethralis muscle. The main aetiology in stress urinary incontinence is due to the urethral support loss. Posterior pubo urethral ligament elongation causes urethral support loss.

FASCIA

The arcus tendineus fascia pelvis is a fibrous band. They extend from pubic bone on right and left side of urethra and vagina.

Ischial spine is their insertion. They provide ligamentous support to the pelvic organs. This support acts like a rope.

Endo pelvic fascia extends from pelvic side walls and arcus tendineus fascia pelvis bilaterally. It extends up to the bladder and the vagina. The bladder and the bladder neck are supported on the posterior aspect by endo pelvic fascia.

According to DeLancy (2001), endo pelvic fascia has three zones to support bladder. Bladder above the cervix is supported by upper zone. Trigone is supported by middle zone. Bladder neck is supported by lower zone.

Pubo cervical fascia is the fibro muscular layer of the anterior vaginal wall. It extends from the pubis anteriorly. It extends up to the uterine cervix posteriorly. The pubo cervical fascia acts like a hammock to support the bladder. The front end of pubo cervical fascia supports the bladder neck and the proximal urethra.

ROLE OF CONNECTIVE TISSUE

Wall of the bladder is made up of,

- Inner urothelium
- Sub urothelial layer

- Detrusor muscle
- Outer serosal layer

All these layers have collagen, elastin, smooth muscle, fibroblasts and blood vessels. The primary function of tension transfer in most of the tissues is provided by collagen.

Detrusor muscle has type 1 and type-III collagen. Urothelial basement membrane has type 1V collagen. The surrounding individual smooth muscle cells also have type 1V collagen. Connective tissue provides bladder wall tissue structure. It also gives tensile strength and resilience. This tensile strength depends on quality of collagen. Types of collagen and its arrangements also play major role in maintaining the tesile strength.

Bladder compliance is affected by changes in collagen type. Endopelvic fascia also contains collagen. Stress urinary incontinent females have changes in the quality collagen. It also depends on type and the quantity of collagen in endopelvic fascia.

The regulation of collagen synthesis depends on,

- Intrinsic factors within individual cell types
- Extrinsic factors like

- ❖ Cytokines
- ❖ Growth factors
- ❖ Mechanical forces

Structural weakness is caused by the progressive alteration of the connective tissue.

In the integral theory, Petros and Ulmsten in 1990 stated that anatomical defects of Stress urinary incontinence are due to connective tissue abnormality. This leads to laxity of anterior vaginal wall. The closure pressure is generated by pubo urethral ligaments, the vaginal hammock and the pubococcygeus muscle. This pressure is not efficiently transmitted by the deficient anterior vaginal wall.

As age increases, there is reduction in the ratio of connective tissue to muscle. Flexibility and remodelling is prevented by collagen cross links. It is stabilized by the collagen molecules. Thus the pelvic floor connective tissue plays a major role in preventing urine leak.

CONNECTIVE TISSUE

Collagen is a fibrous protein. 30% of total body protein is made is made up of collagen. Fibroblasts produce collagen. The

tensile strength of skin, tendons and bones are provided by collagen. Totally 19 different types of collagen were identified in our body. Main structural component of epithelial tissues are type 1 and type-III.

MAIN TYPES OF COLLAGEN:

Tissue distribution	Types of collagen
Bone, tendon, skin, dentin, ligament, fascia, arteries	Type-I
Hyaline cartilage	Type-II
Skin, arteries, uterus	Type-III
Basement membrane	Type-IV

TYPE-I COLLAGEN

Most abundant collagen found in human body is type 1 collagen. Bone, tendon and skin have type 1 collagen. Dentin, ligaments, fascia and arteries also contain type 1 collagen. It is involved in many diseases like fibrosis, osteoporosis, malignancy and atherosclerosis. Various pathological conditions are diagnosed by type 1 collagen analysis.

Type-I collagen contains α chain. This chain has three polypeptide chains. Each chain is made up to 1050 amino acids. The three polypeptide chains joined together. These chains join

together to form triple helical structure. It is a right handed triple helix.

Type-I collagen is a hetero trimmer. It is made up of two α1 chains and one α2 chain. Length of triple helix is 300nm. The diameter is 1.5 nm. Glycine, proline and hydroxylproline joined together to form type 1 collagen.COL1A1 gene encodes type 1 collagen chain.

Type-I collagen fibrils are packed side by side in parallel bundles. They are several micrometers length. 50nm is the diameter. Type 1 collagen fibrils have enormous tensile strength. Collagen-1 can be stretched without being broken. It withstands enormous forces. Strength of type 1 collagen is more than steel.

TYPE-III COLLAGEN

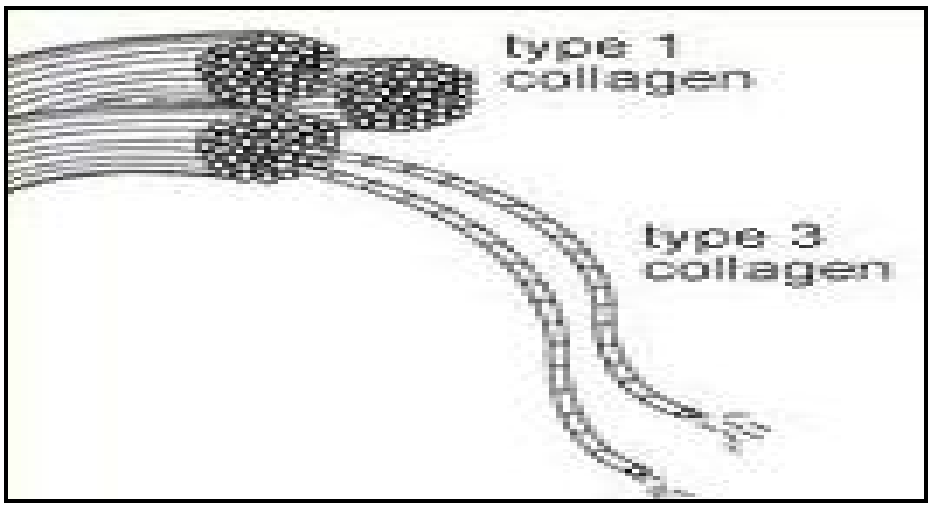
Type-III collagen is a fibrillar collagen. Skin, lung and arteries contain type-III collagen. The second most collagen found in human body is type-III collagen.COL3A1 gene in chromosome 2 encodes collagen-III chains

Type-III collagen is a homotrimer. It is made up of three α1 chain. The chains are interlinked by disulphide bonds. Its elastic properties are due to disulphide bonds.

DIFFERENCES BETWEEN TYPE-I AND TYPE-IIII COLLAGEN:

	Type 1 collagen	Type 111 collagen
Gene	COL1A1	COL3A1
Structure	heterotrimer	homotrimer
Mechanical stability	More	Less
Elasticity	More	Less
Strength	More	Less
Toughness	More	Less

Figure - Picture of type-I and type-III collagen



Hence type-I collagen gives more support compared to type-III collagen. Definitely changes in collagen types have a role in the aetiology of urine leak.

PREDISPOSING FACTORS

Large numbers of female population have urine leak problems. Prevalence of urine leak ranges from 8 – 30%. Urethral

hyper mobility and intrinsic sphincter deficiency are the main defect seen in stress urinary incontinence.

Various aetiological factors cause changes in pelvic support. Endo pelvic fascia in the mid urethra plays a major role in the prevention of urine leak. By its lateral attachment to arcus tendineus fascia pelvis, it gives support.

Urethral stability is increased by endo pelvic ligaments. Levator ani muscles also give stability. They provide suspension and cushion effect. Urethral closure pressure is affected, whenever there is derangement in the supporting structures of urethra.

Collagen Matrix

Women with congenital connective tissue disorders are found to have high prevalence of stress urinary incontinence due to altered collagen support. Various studies have shown that the following changes in connective tissue of the pelvic support leads to stress urinary incontinence

- ❖ Decreased collagen production
- ❖ Increased nascent collagen degradation
- ❖ Increased collgenolytic activity

- ❖ Increased matrix metalloproteinase activity
- ❖ Decreased protein levels in total collagen
- ❖ Reduction on type 1/ 111 ratio
- ❖ Reduction in total collagen content
- ❖ Reduction in elastin levels
- ❖ Increased elastolysis activity
- ❖ Increased proteolysis activity

Alterations in genes

Various studies have shown the role of genetics. They are as follows,

- ❖ Increased incidences
- ❖ Dominant mode of inheritance
- ❖ Increased incidence in nulliparous women

Age

Aging is associated with pelvic floor dysfunction. It is due to changes in hormonal levels following menopause.

Ethnic variation

Women of Hispanic group and African American women found to have increased incidence of stress urinary incontinence.

Birth injury

Birth injury causes,

- ❖ Injury to the connective tissue support
- ❖ Vascular damage to the pelvic structures
- ❖ Injury to the pelvic nerves
- ❖ Injury to the muscles

There by strength of supportive tissue is lost. Hence stress urinary incontinence results.

Obesity

Body mass index more than 30kg/m2 found to have increased incidence of stress urinary incontinence.

Life style problems

The following factors are inconsistancely associated with stress urinary incontinence

- ❖ Depression

- ❖ Work stress
- ❖ Increased water intake

INVESTIGATIONS

The investigations done while evaluating the stress urinary incontinent women are as follows,

1. Voiding diary and pad tests

- ❖ To find the severity of symptoms
- ❖ To know the incontinence episodes.
- ❖ Complete a minimum of 3 days of the diary - both working and leisure days should be noted

2. Urine analysis

Urine should be analysed to rule out associated causes like hematuria, pyuria, proteinuria, glycosuria.

3. Urine culture and sensitivity

Urinary tract infection can cause urinary incontinence. It may cause detrusor over activity. A mid stream urine specimen is used for urinalysis. Urine microscopy and culture also should be done.

4. Blood urea nitrogen and serum creatinine

To assess renal function, blood urea nitrogen and serum creatinine should be tested.

5. Ultra sonogram of abdomen pelvis with post void residual urine

- To assess the upper tracts
- To assess the amount of residual urine in the bladder

6. Urodynamic evaluation

Urodynamic evaluation is not routinely done in cases if stress urinary incontinence. Urodynamic evaluation is must in cases of,

- Mixed urinary incontinence
- Previous history of surgery for stress urinary incontinence
- Detrusor over activity
- Bladder outlet obstruction
- To rule out neurogenic bladder

7. Q tip test

Angle of deviation more than 30 degree is suggestive of stress urinary incontinence

8. Bonney's test (cough stress test) and Bladder elevation test (Marshall Test)

These tests are usually done to find urine leak during stress. These tests are also done to predict the success of bladder neck suspension surgeries. Since this test may occlude the urethra and

may give false results. Hence these tests are not predictors of successful outcomes of surgeries.

9. Cystoscopy

To exclude other lesions in bladder and urethra, urethro cystoscopy should be done.

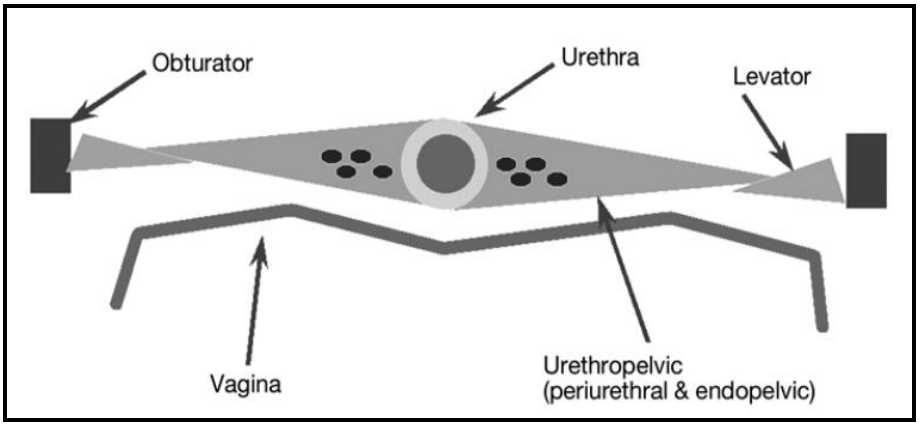
10. MRI

At present, there is no recommendation for MRI.

IMPORTANCE OF PERI URETHRAL VAGINAL TISSUE

Pubo cervical fascia which is a part of endo pelvic fascia.Along with levator ani, it gives support to the urethra. Collagen is the main connective tissue component of endo pelvic fasia.

Figure: Picture of periurethral and endopelvic fascia



Various studies have shown that para urethral tissues and peri urethral vaginal tissues are analogous to pubo cervical fascia (endopelvic fascia). Hence analysis of peri urethral vaginal tissues gives idea about the nature of endopelvic fascia.

It is very easy to take biopsy from peri urethral vaginal tissues without any open surgeries. Hence analysis of peri urethral vaginal tissues for collagen gives an idea about the collagen status of endopelvic fascia.

MATERIALS AND METHODS

TITLE OF THE STUDY

Analysis of collagen status in women with genuine stress incontinence

PERIOD OF STUDY

March 2012 – February 2013

STUDY DESIGN

Case control study

PLACE OF STUDY

The study was conducted in the Department of Urology and Pathology, Madras Medical College and Rajiv Gandhi Government Hospital, Chennai – 3

ETHICAL CLEARANCE

The institutional ethical review board at our hospital approved the study (No:19022012)

INCLUSION CRITERIA

Cases:

Female patients with stress urinary incontinence

Controls

Female patients with calculus diseases

EXCLUSION CRITERIA:

Cases:

Patients with

- ❖ Mixed urinary incontinence
- ❖ Urge urinary incontinence
- ❖ Pregnant women
- ❖ Patients with known collagen disorder
- ❖ Patients with uterine prolapsed
- ❖ Patients attained menopause

Controls

- ❖ Patients previously intervened for stress urinary incontinence
- ❖ Pregnant women
- ❖ Patients with known collagen disorder

METHOD OF STUDY

All the patients are informed about the procedure. After explaining details of the study, consent form is filled with signature. All details were recorded in a proforma as an outpatient procedure. Analysis was done with the collected details prospectively.

PATIENT EVALUATION

All the patients were enquired regarding menstrual history, parity and previous surgical details. All the patients were undergone abdominal examination, per vaginal examination, cystoscopy and urodynamic evaluation.

Biopsies for collagen analysis were taken from all cases and all continent controls. The biopsy was taken from the periurethral vagina following application of lignocaine cream. The site of biopsy was 1 cm lateral to the urethra in its lower third.

The samples were immediately fixed in formalin for a period of 24 hrs and embedded in paraffin wax. 5 µm sections were mounted and stained with Eosin and Haematoxylin for histological assessment. Immunohistochemical analysis was done in all biopsy specimens.

The biopsy materials were reviewed by experienced pathologists. The reviewers were blinded to the patients' clinical details and analyzed the biopsy material independently for collagen content and the ratio of collagen-I and collagen-III.

ANTIBODIES

The primary and secondary antibodies used in this study were monoclonal antibodies against collagen-I (Abcom Ltd) and

collagen-III (Biogenix Ltd). Staining pattern difference on microscopic examination which was shown by the highest dilution was used for analysis.

IMMUNOHISTOCHEMICAL EXAMINATION

The basic steps followed for the IHC protocol are the tissue fixing and embedding, the section cutting and mounting, the section deparaffinising and rehydrating, retrieval of antigen, staining with Immunohistochemical stain, if needed counterstaining , dehydration and stabilization with mounting medium and examination of the stained areas under the microscope.

IMAGE ANALYSIS

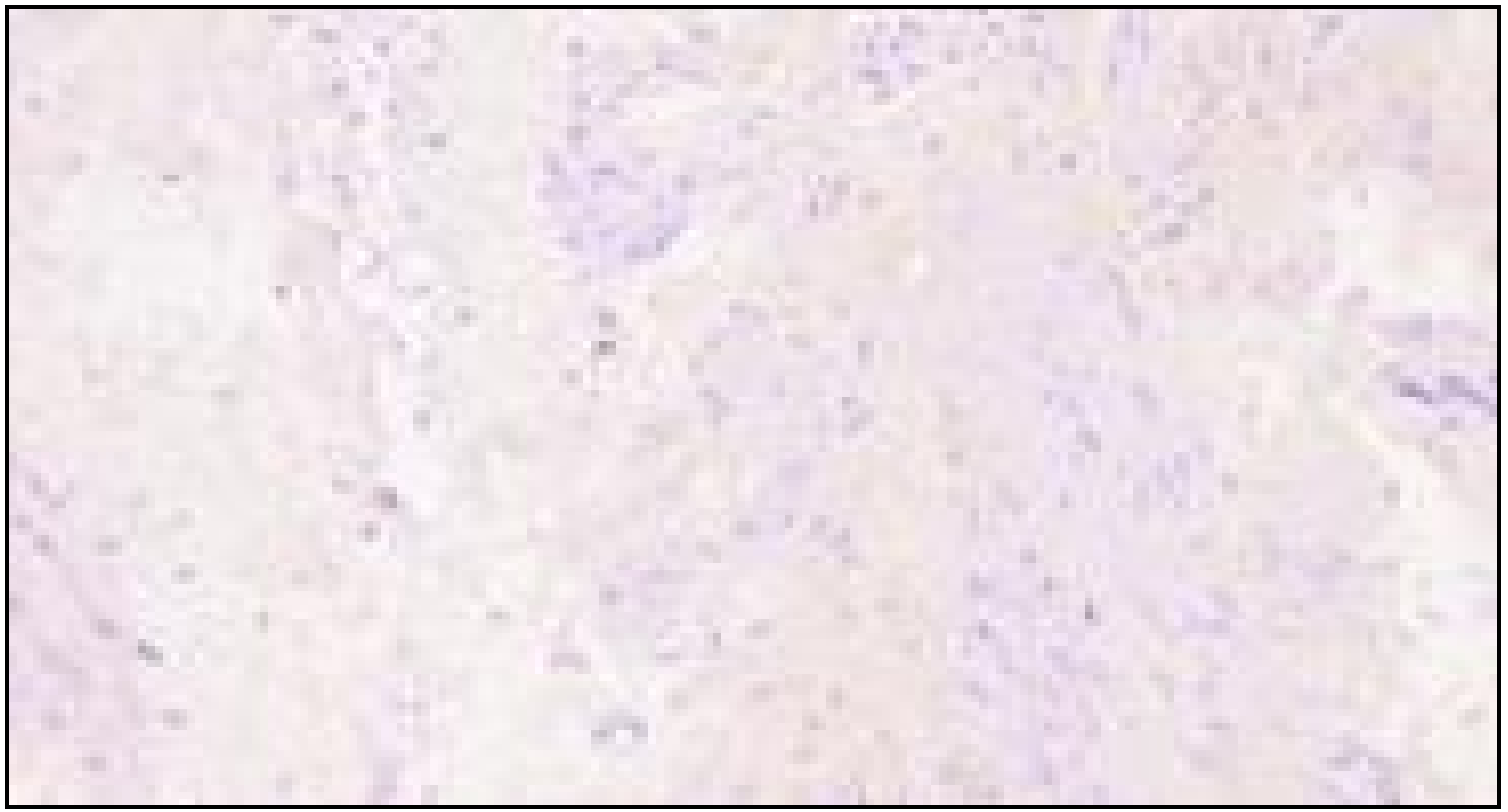
Colour video camera and microscope were used for capturing the images of the tissue sections. 10 randomly selected fields from each slide were used for analysis. The blood vessels and the smooth vessels in the field were not included in the study. Outlining of stained collagen area was done and the percentage of stained area was measured. From the measurements, Microsoft excel programme was prepared for statistical analysis

STUDY ANALYSIS

The statistical package for the Social Sciences, version 18.0.2 (SPSS Inc, Chicago. IL, USA) was used for the statistical analysis. A p value equal to or below 0.05 was considered significant.

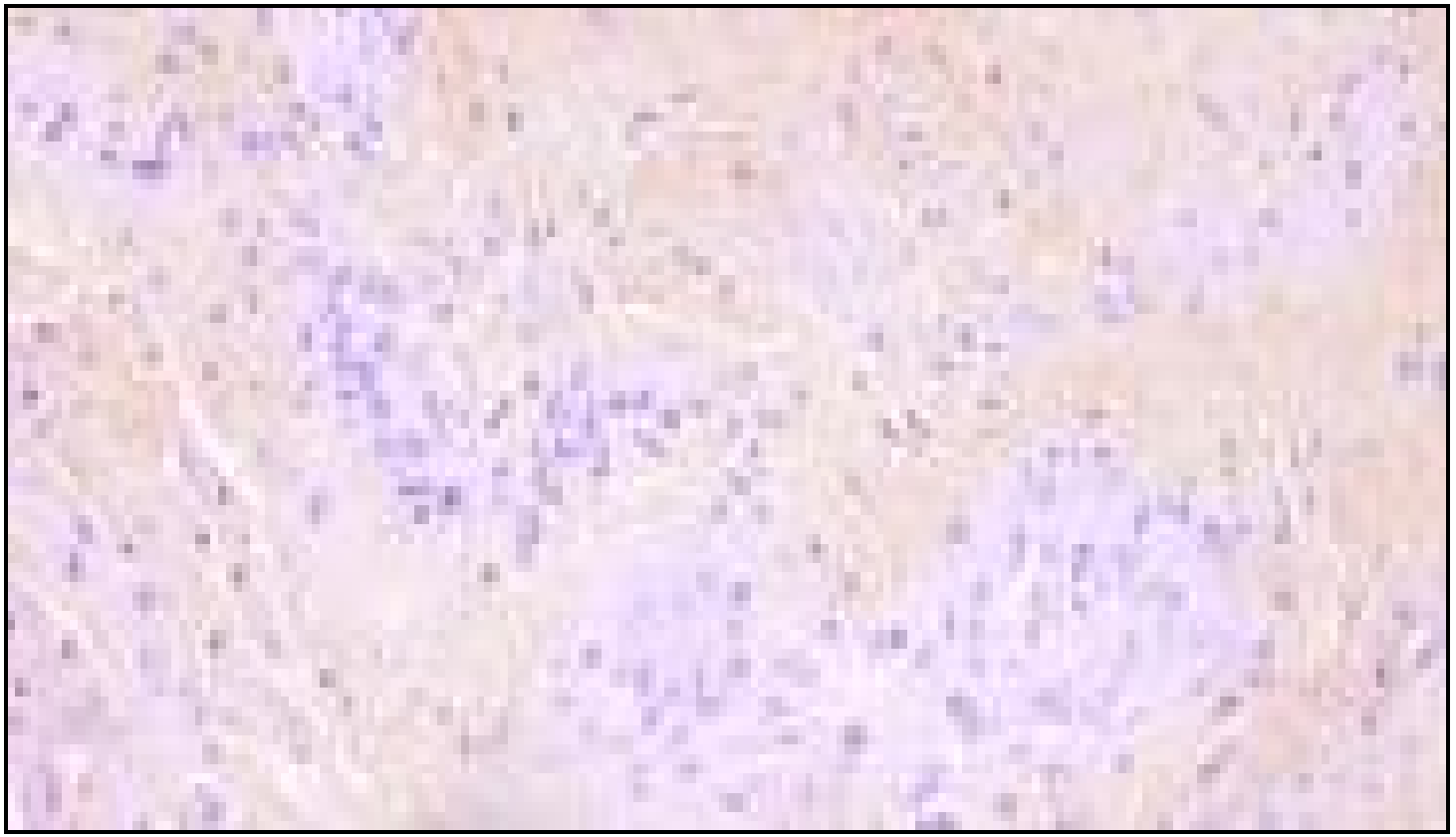
IMMUNOHISTOCHEMICAL STAINING OF COLLAGEN 1

Cases



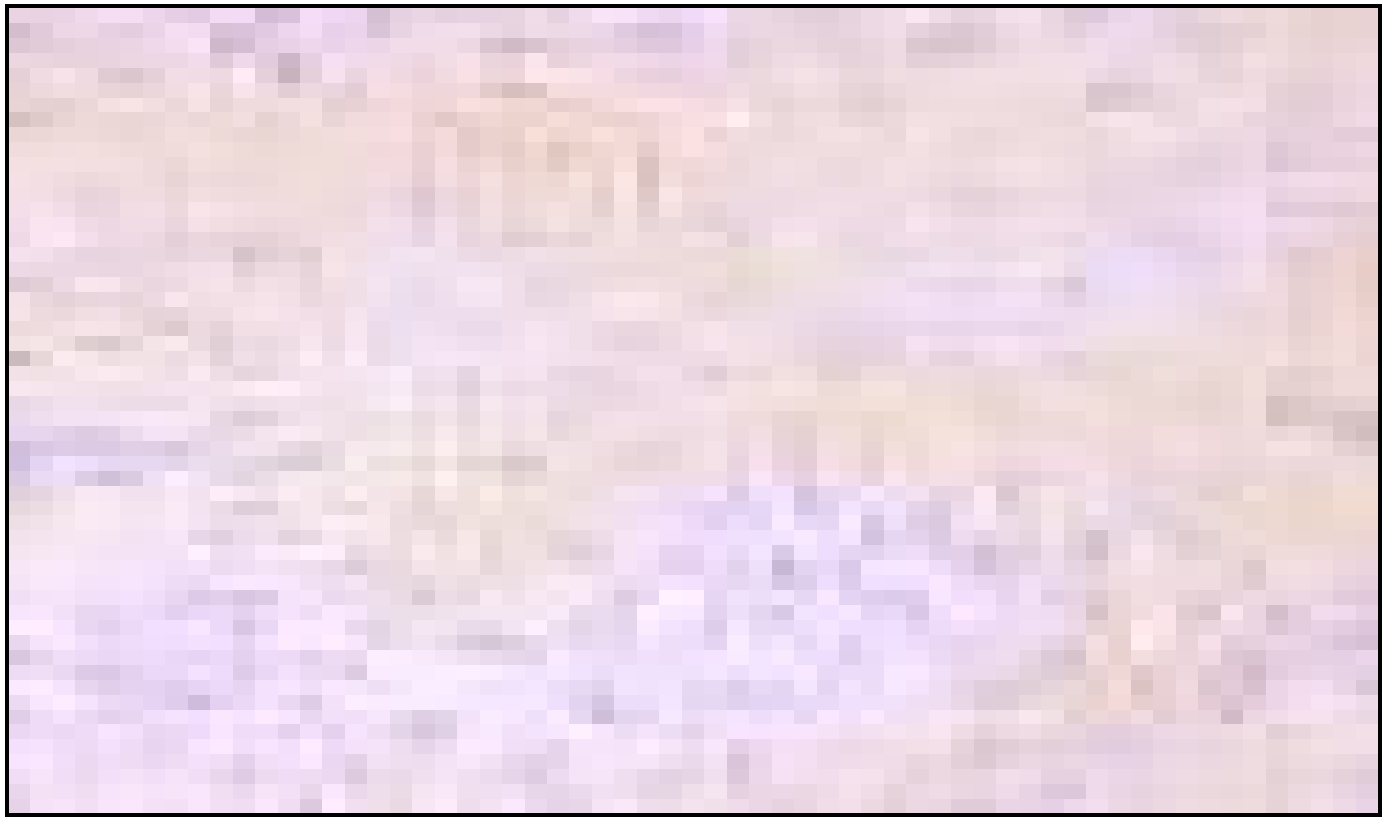
IMMUNOHISTOCHEMICAL STAINING OF COLLAGEN 1

Controls



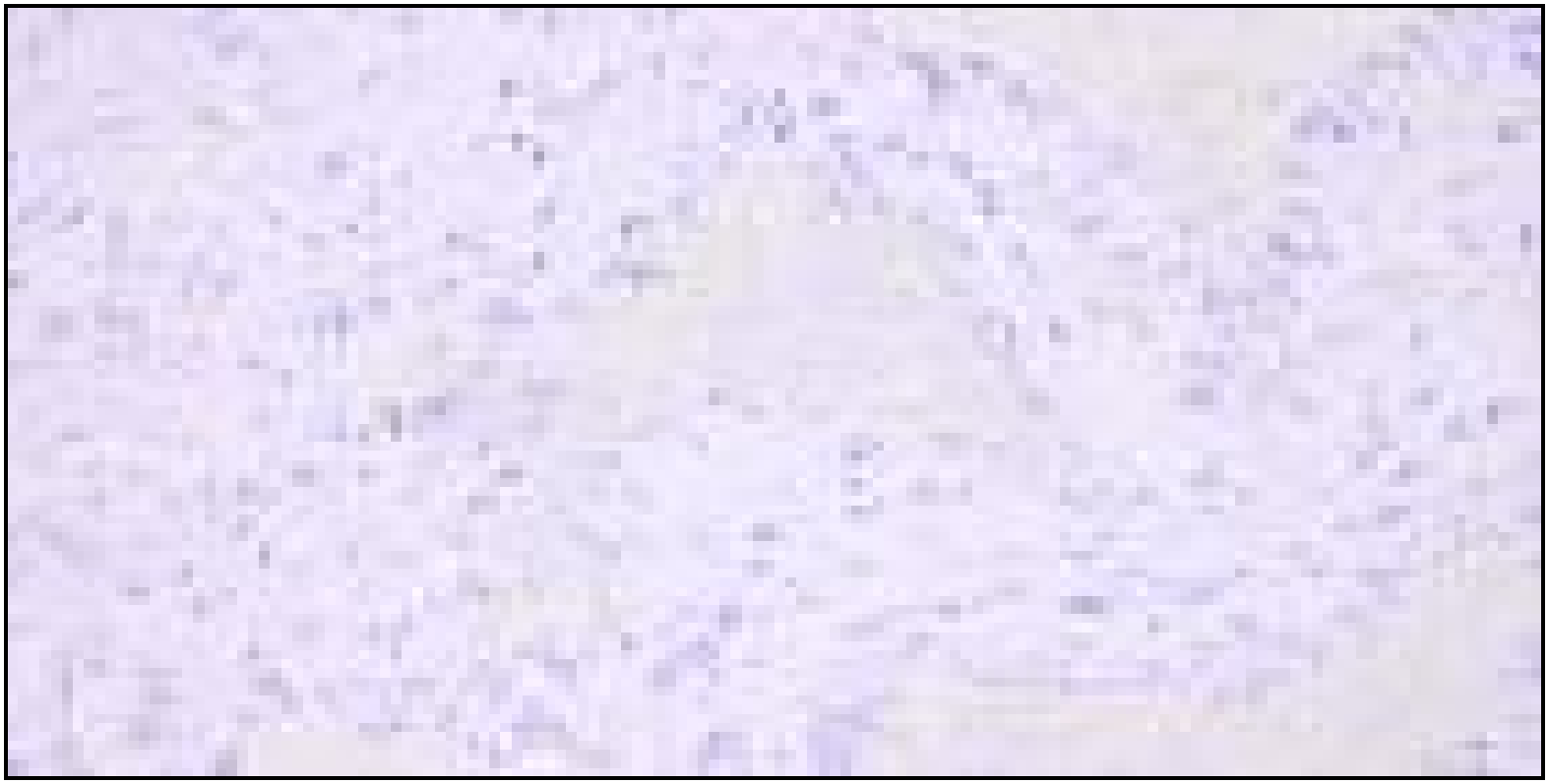
IMMUNOHISTOCHEMICAL STAINING OF COLLAGEN 111

Cases



IMMUNOHISTOCHEMICAL STAINING OF COLLAGEN 111

Controls



OBSERVATION AND RESULTS

DESCRIPTIVE STATISTICS

Our study consists of 50 patients. 25 patients were cases who had urodynamically proven genuine stress urinary incontinence and 25 controls without stress urinary incontinence.

The patient characteristics in the two groups are shown in the table 1

Table-1: Patient characteristics Chi-Square test to compare the proportions between groups

		Group				Total	
		Control		Cases			
		N	%	N	%	N	%
Parity	Nil	4	16.0	3	12.0	7	14.0
	Primy	4	16.0	8	32.0	12	24.0
	Multi	17	68.0	14	56.0	31	62.0
MODE OF DELIVERY	Nil	4	16.0	2	8.0	6	12.0
	Normal	14	56.0	14	56.0	28	56.0
	LSCS	7	28.0	9	36.0	16	32.0
DM	No	22	88.0	20	80.0	42	84.0
	Yes	3	12.0	5	20.0	8	16.0
HTN	No	22	88.0	21	84.0	43	86.0
	Yes	3	12.0	4	16.0	7	14.0
Total		**25**	**100.0**	**25**	**100.0**	**50**	**100.0**

Table 2 – Patients characteristics

Chi-Square Tests (Fisher's Exact test)	P-Value
Parity * Group	0.438 (NS)
MODE OF DELIVERY * Group	0.668 (NS)
DM * Group	0.702 (NS)
HTN * Group	0.995 (NS)

P – Value

NS – Not significant

HS – Highly significant

Among the cases 4 were nulliparous women and 3 women were nulliparous in control arm. 4 women were primyparous in cases group and 8 were primyparous in control group. 17 women had multiple deliveries in cases arm and 14 women had multiple deliveries in control group. Among the cases 14 women had labour natural and 14 women had labour natural in control group. 7 women in cases group had LSCS and 9 women in control arm had LSCS. All these characteristics were found to be statistically insignificant.

Figure 1 – Parity distribution between cases and controls

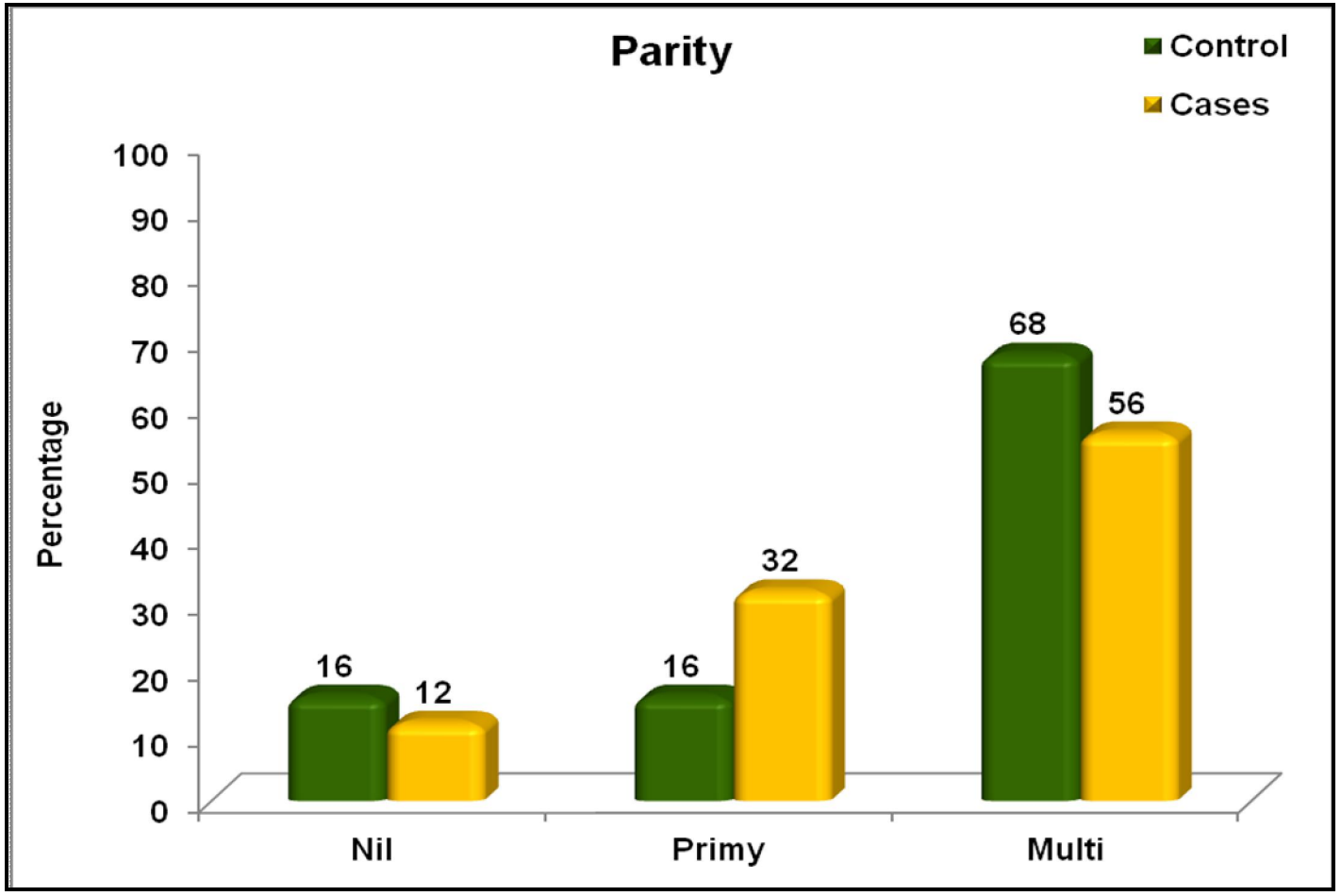
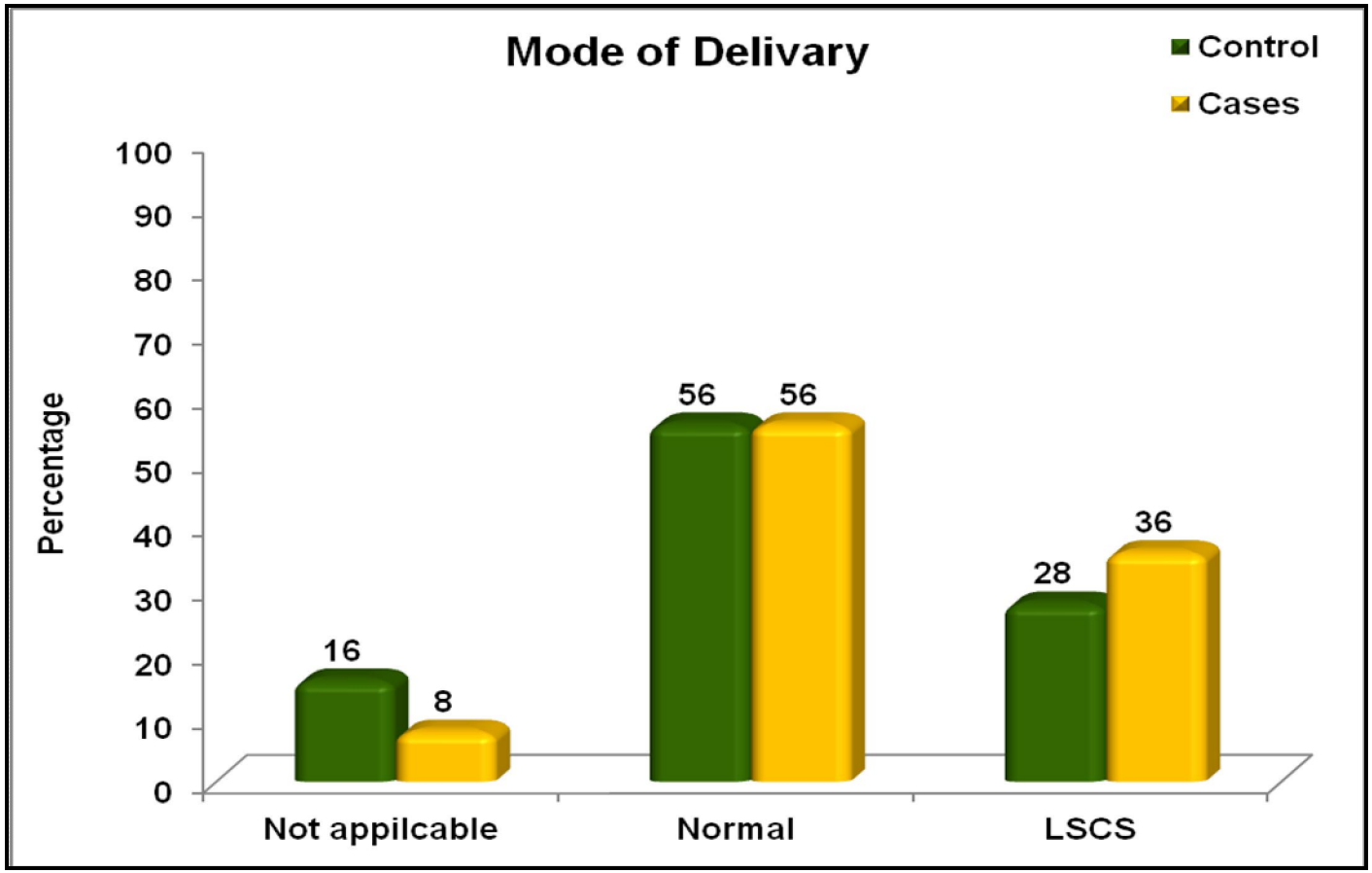


Figure 2 – Mode of delivery between cases and controls



Among the cases 3 women had diabetes and 4 women in the control arm had diabetes. 3 women in cases had hypertension and 4 women in the control group had hypertension. All these characteristics were found to be statistically insignificant.

Figure 4 – Distribution of diabetes and hypertension between cases and control

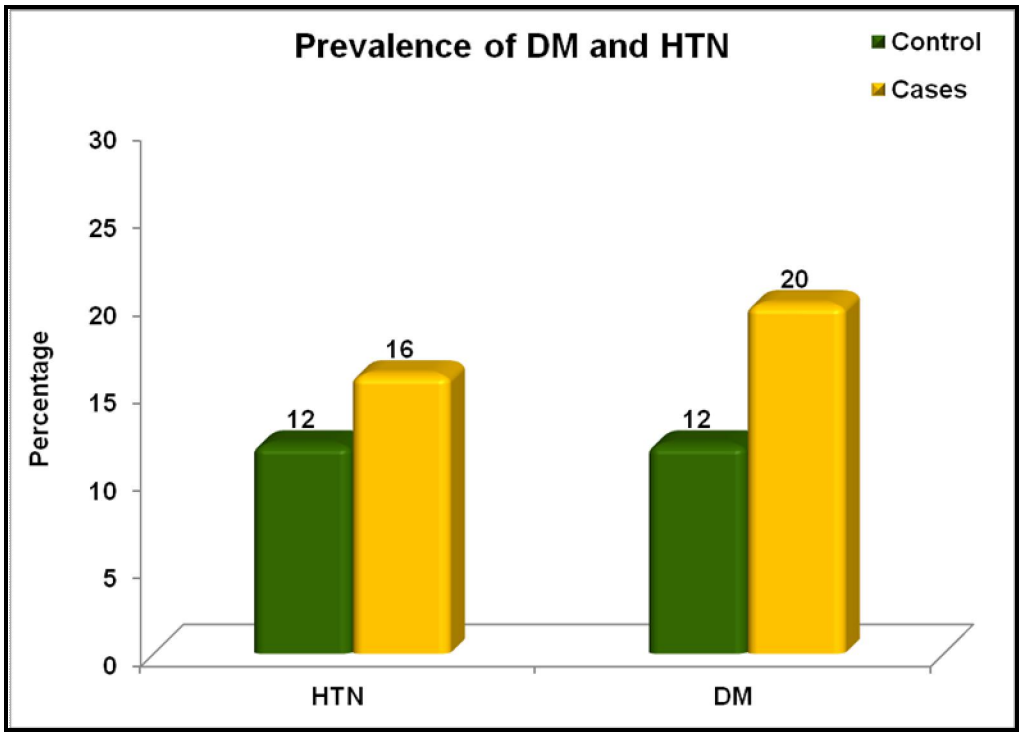


Table-3 - Patient characteristics Independent samples T-Test to compare the mean values between cases and controls

Variables	Group	N	Mean	Std. Dev	P-Value
Age	Control	25	39.44	5.973	0.645
	Cases	25	40.16	4.955	
Weight	Control	25	66.84	8.994	0.017
	Cases	25	60.44	9.283	
Daily water intake in litres	Control	25	2.720	0.5220	0.244
	Cases	25	2.540	0.5575	
Voids/day	Control	25	5.48	0.918	0.884
	Cases	25	5.52	1.005	
Voids/night	Control	25	0.52	0.872	0.131
	Cases	25	0.88	0.781	

The mean age of the control was 39.4 years and the mean age of the cases was 40.2 years. The mean body weight in the cases group was 60.4 kgs and in the control arm was 66.8 kgs. All these characteristics were not statistically significant.

Figure 5 – Age distribution between cases and controls

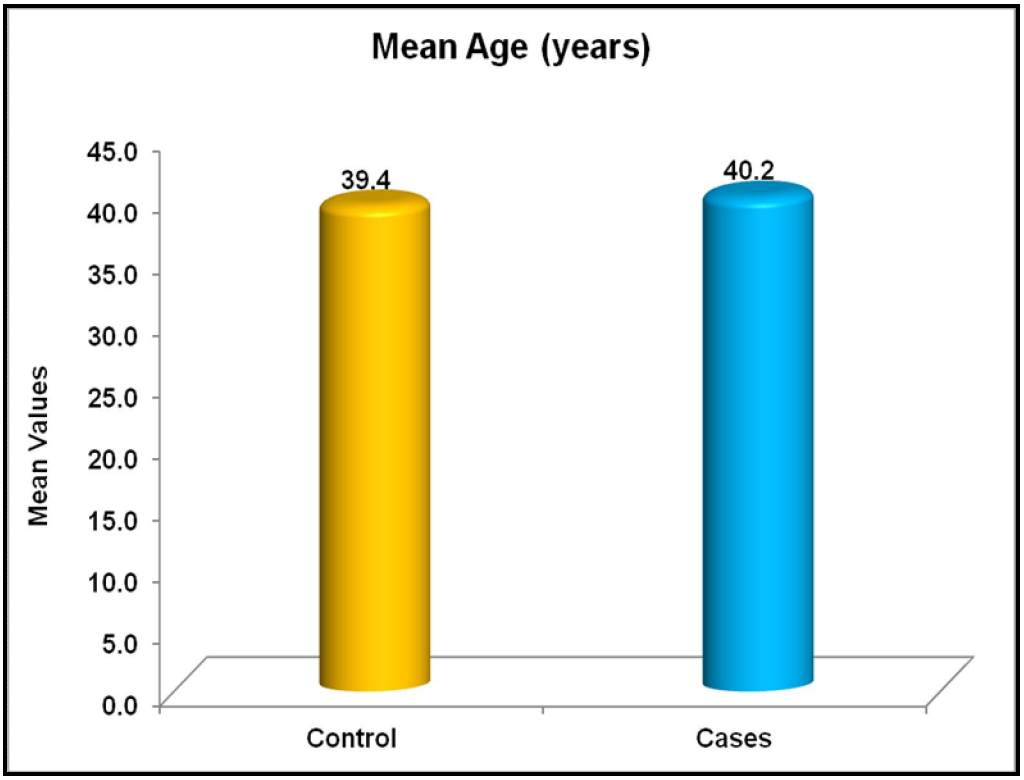
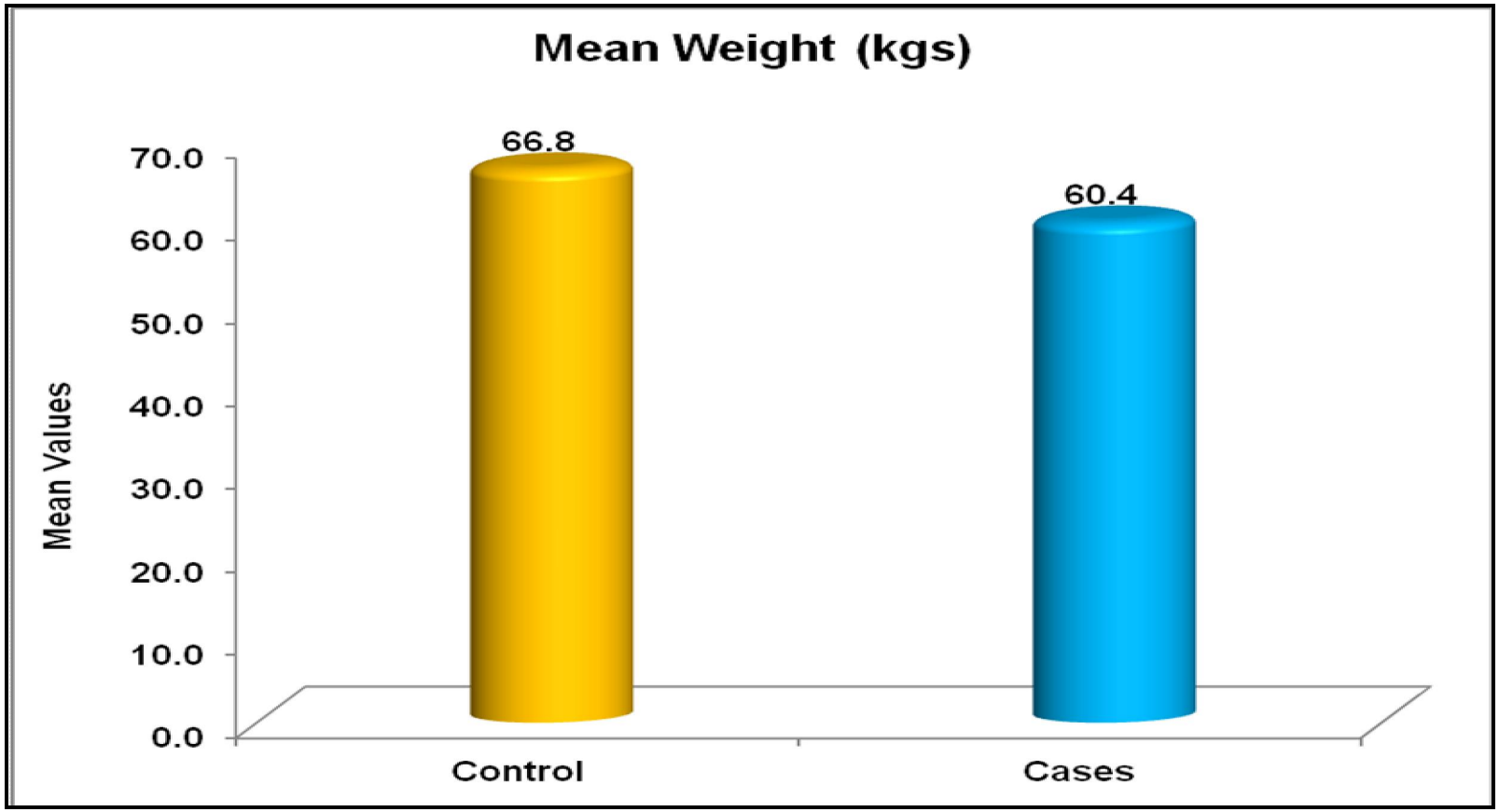


Figure 6 – Body weight distribution between cases and controls



The mean water intake in the cases group was 2.5 litres per day and in the control arm was 2.7 litres per day. The mean voids per day were equal in both the cases and control group and was about 5.5 voids per day. The mean voids per night in cases group were 0.9 and in the control arm were 0.5. All these parameters were not statistically significant.

Figure 7 – Daily water intake between cases and controls

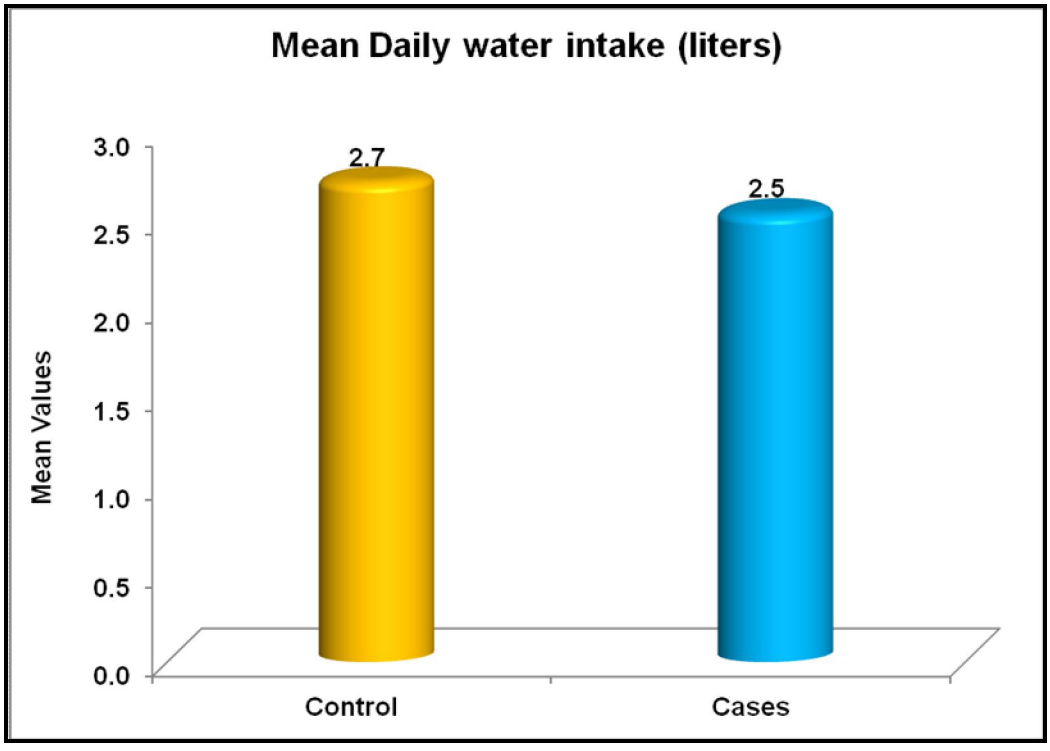


Figure 8 – Distribution of mean voids per day between cases and controls

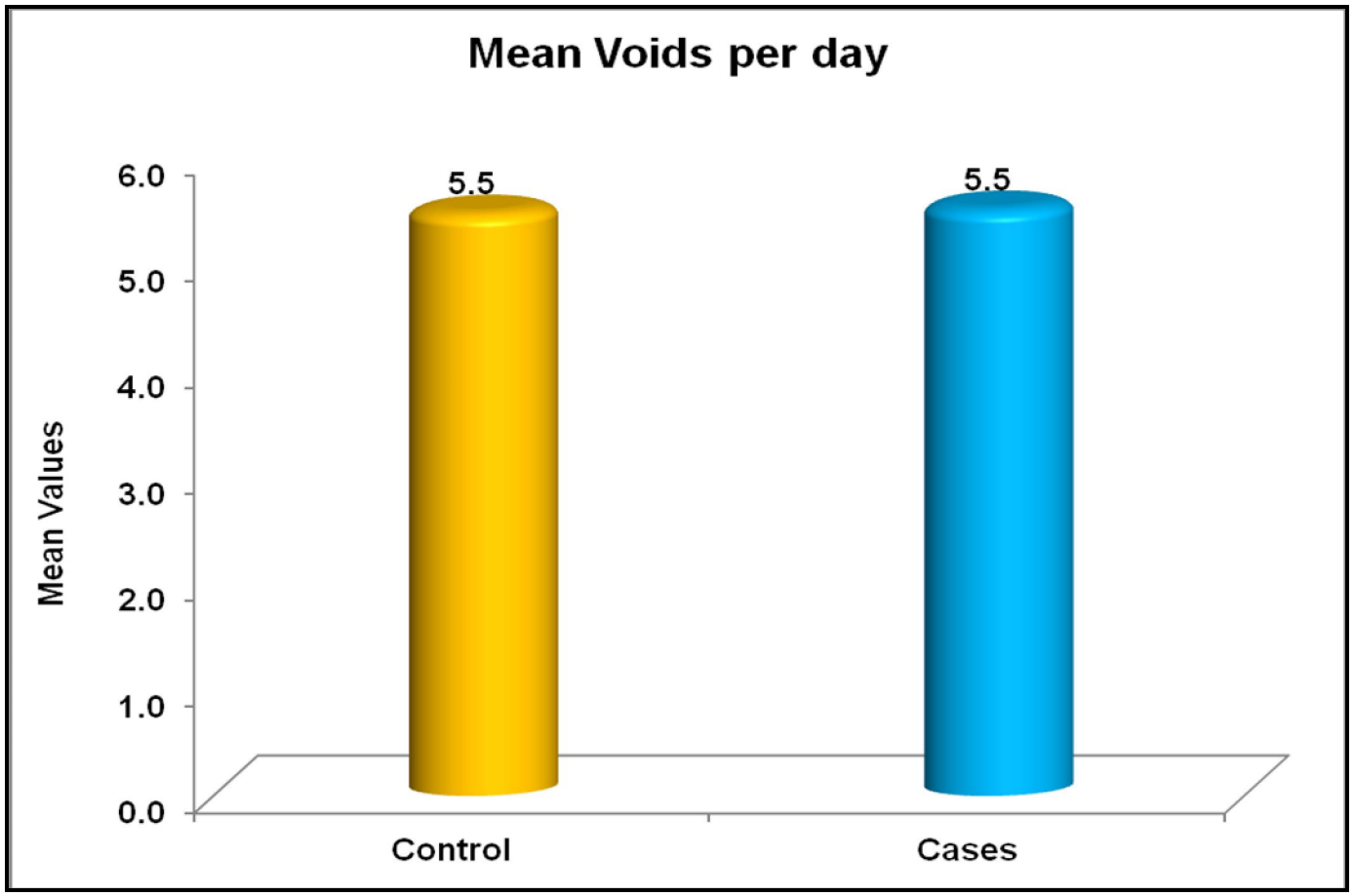


Figure 9 – Distribution of mean voids per night between cases and controls

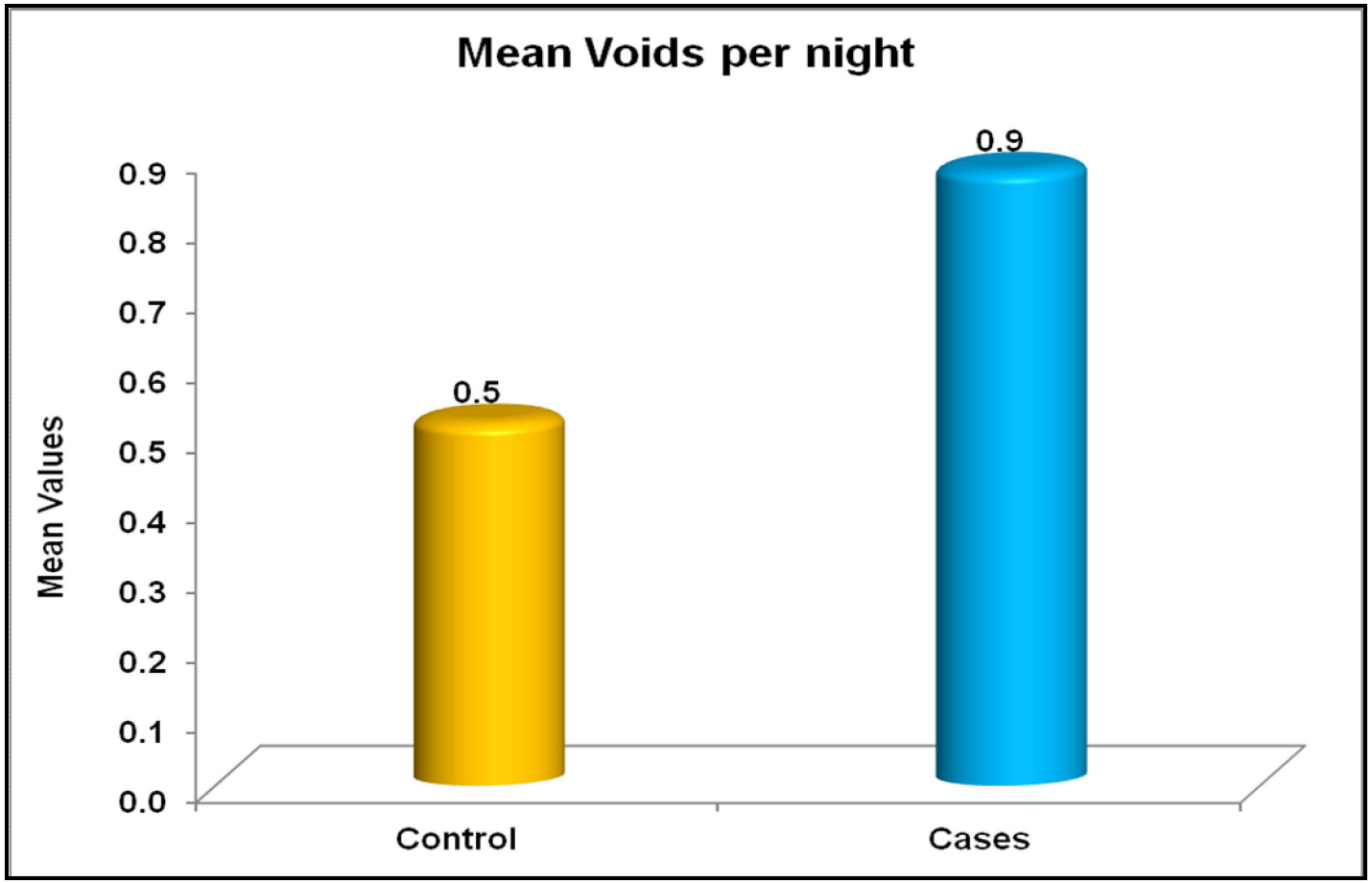
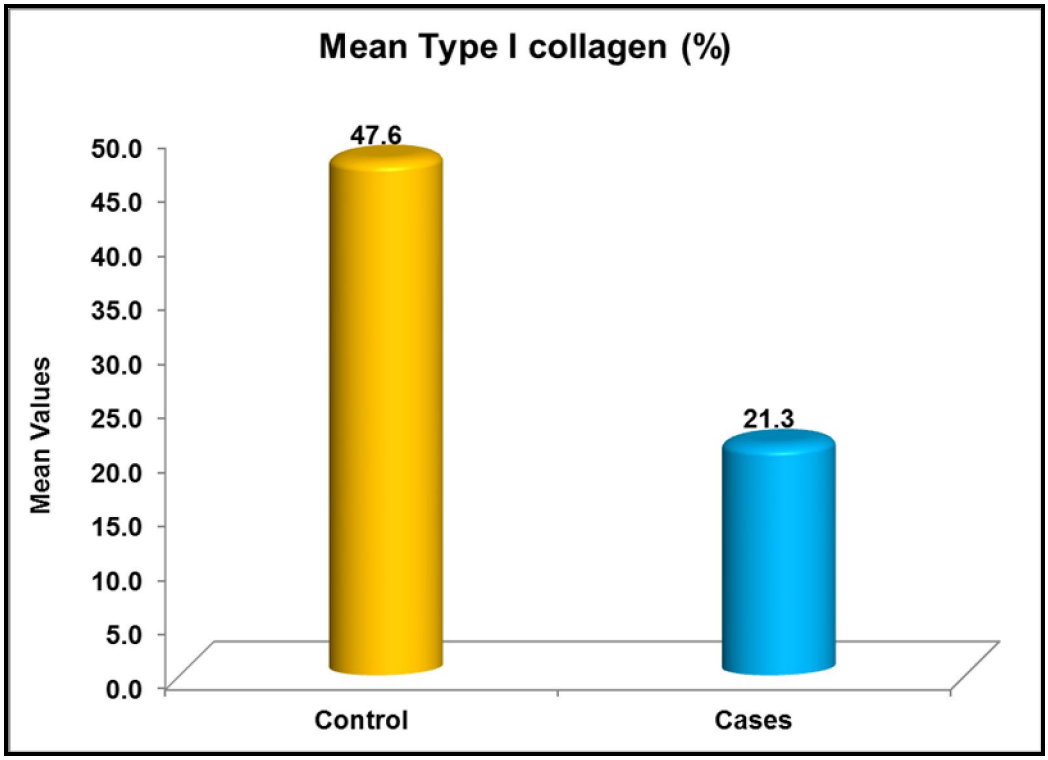


Table 4 - Comparision of collagen 1 and collagen 111 between cases and controls Independent samples T-Test to compare the mean values between cases and controls

Variables	Group	N	Mean	Std. Dev	P-Value
Type I collagen (%)	Control	25	47.56	7.258	0.001
	Cases	25	21.32	7.034	
Type III collagen (%)	Control	25	18.56	5.938	0.001
	Cases	25	46.36	7.588	
Collagen I/III ratio	Control	25	2.79	0.904	0.001
	Cases	25	0.46	0.158	

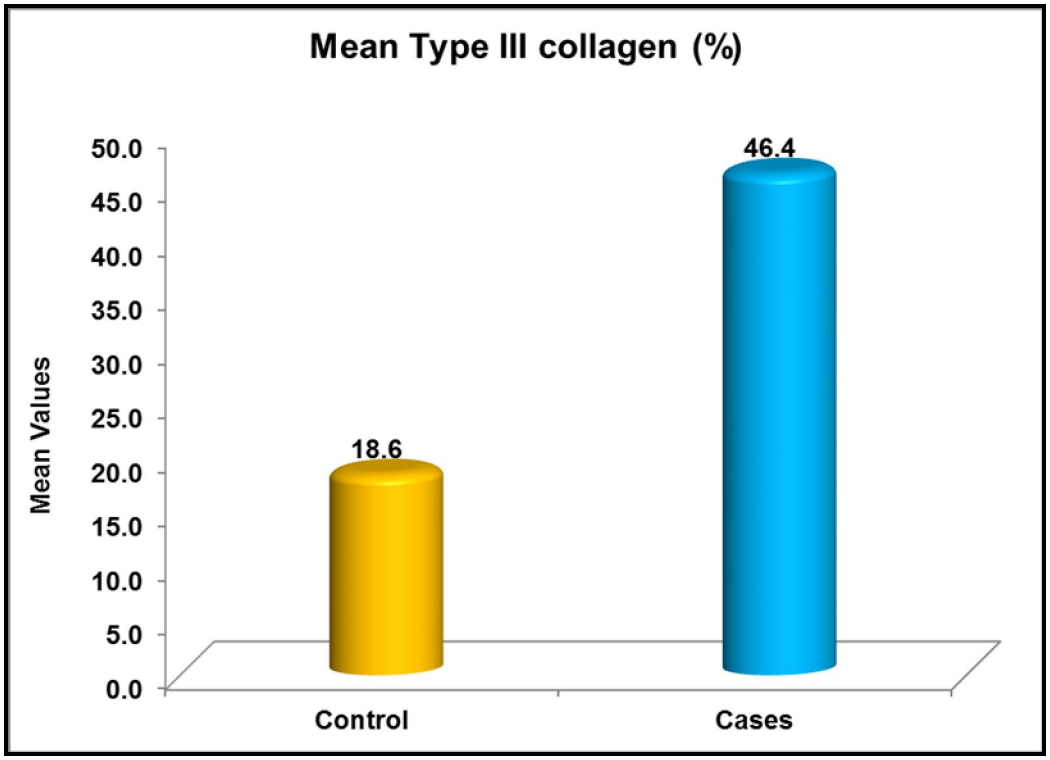
The mean percentage of collagen 1 staining in cases arm was 21.3% and in the control arm was 47.6%. These findings showed that collagen 1 was higher in the control arm (women without stress urinary incontinence) compared to cases arm (women with stress urinary incontinence). The difference was statistically significant with P- value 0.001.

Figure-11: Mean distribution of collagen 1 between cases and controls



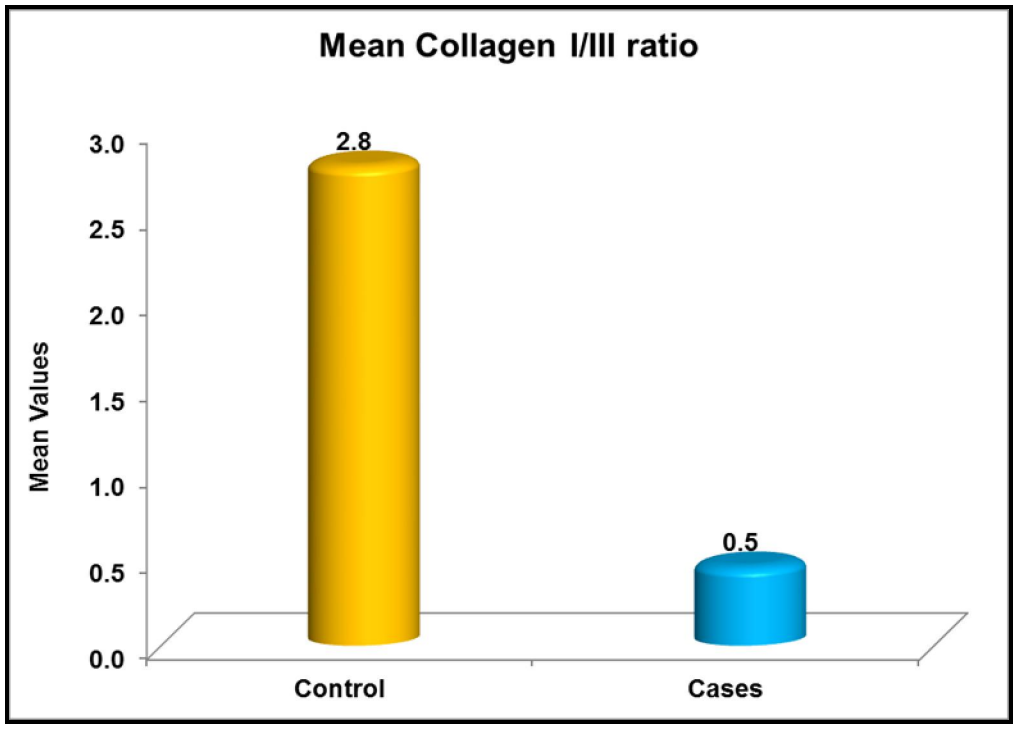
The percentage of collagen-III staining in cases arm was 46.4% and in the control arm was 18.6%. These findings showed collagen-III was higher in cases arm (women with stress urinary incontinence) compared to control arm (women without stress urinary incontinence). The difference was statistically significant with P- value 0.001.

Figure 11 – Mean distribution of collagen-IIII between cases and controls



The mean value of collagen 1/111 ratio in cases arm was 0.5 and in the control arm was 2.8. These findings showed that collagen 1 was higher in the control arm (women without stress urinary incontinence) compared to cases arm (women with stress urinary incontinence). These findings also showed collagen 111 was higher in cases arm (women with stress urinary incontinence) compared to control arm (women without stress urinary incontinence). The difference was statistically significant with P-value 0.001

Figure-12: Mean distribution of collagen 1/111 ratio between cases and controls



DISCUSSION

Changes in collagen metabolism in fertile women are associated with stress urinary incontinence. Impaired mechanical function from altered extra cellular matrix which is more rigid, leads to stress urinary incontinence.

Falconer et al tested the organisation of collagen and analysed the diameter of collagen fibrils in the paraurethral connective tissue and found that larger collagen fibrils and lower proteoglycon and collagen ratio was associated with stress urinary incontinence.

Liapiasa et al in his study has shown alterations in the type 111 collagen around the urethra in stress urinary incontinent women .Paul Abram's et al found alteration in collagen cross link in the biopsies of peri urethral vagina. Wong et al found decreased collagen content in the uterine cervix and there was no significant relationship between collagen changes and age, parity and body mass index.

Harmani et al in his The Temple study suggested that changes in collagen leads to pelvic floor disorder. This altered collagen metabolism acts as an intrinsic factor and aging, obesity and multiparity act as a additive factors only.

Abnormal collagen synthesis or imbalance between degradation and synthesis leads to pelvic support disorders. The type of collagen, synthesis rate, organisation of collagen, collagen cross linking and collagen remodelling determines the supportive function of ligaments and fascia.

Collagen 1 and 111 has different biomechanical characters and tissue function depending on their concentration. Connective tissue mechanical strength depends in collagen1. Elastic nature of tissue and extensibility of tissue depends on collagen 111. The ability of the ligament and fascia to support the pelvic organs depends on the type of collagen and collagen bundle amount. Alteration in collagen 1 and 111 concentration and collagen1/111 ratio causes decreased tissue mechanical strength and leads to stress urinary incontinence. Since collagen regeneration is lost in adults, alteration in collagen concentration leads to loss of connective tissue support and causes stress urinary incontinence.

In our study the cases and the controls were analysed with regard to percentage of collagen1, collagen 111 and collagen 1/111 ratio from periurethral tissue. This periurethral tissue contains pubocervical fascia which is a part of endopelvic fascia. Endopelvic fascia is the major supporting tissue of the bladder and urethra.

Our study demonstrates a highly significant increase in collagen111 in cases compared with controls. Mean percentage of type 111 collagen in cases is 46.4% compared to 18.6% in controls.

Percentage of collagen type 1 is only 21.3% in cases compared to 47.6% in controls. This suggests stress urinary incontinent women have significant changes in their collagen amount in the periurethral tissues which is analogous to endopelvic fascia. Ratio of collagen1/111 is altered in cases compared to control group. By this, it is evident that less supportive collagen is present in periurethral tissues (endopelvic fascia). Hence collagen type changes along with changes in collagen1/111 ratio makes the periurethral tissues (endopelvic fascia) weak in stress urinary incontinent women.

Table 5 - Comparison of collagen status between our study and Kean et al

Analysis	Our study	Kean et al
Percentage of type 1 collagen	21.3%	29%
Percentage of type 111 collagen	46.4%	39.7%
Collagen 1/111 ratio	0.5	0.8

Our study is compared with the study done by Keane et al and the comparative results are given in the chart (table 7). Percentage of type 111 collagen in cases in our study is 46.4% compared to 39.7% in the study by Keane et al. The percentage of type 1 collagen in cases in our study is 21.3% compared to 29% in the study by Keane et al.

Our study has few limitations. The study comprises of small group of patients. Other extra cellular matrix components are not analysed. However the study was done with the belief that collagen is the major structural back bone of endopelvic fascia.

The study has to be done routinely to predict the collagen changes in the periurethral tissues in general populations. Only when these shortcomings are corrected, peri urethral collagen analysis found to be a good diagnostic tool for the women with stress urinary incontinence to decide further management.

CONCLUSION

The distribution of collagen-I and collagen-III between stress urinary incontinent women and continent control women is found to be statistically significant.

These collagen alterations are not influenced by age, parity, mode of delivery, body weight and presence of hypertension and diabetes.

Although these data are statistically significant, large study is needed for future usage as diagnostic tool and to discover the pathophysiology of stress urinary incontinence.

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APPENDIX-I

INFORMED CONSENT FORM

Title of the study:

ANALYSIS OF COLLAGEN STATUS IN WOMEN WITH GENUINE STRESS INCONTINENCE

Name of the Participant:

Name of the Principal Investigator:

Dr. HEMALATHA. K

Name of the Institution:

Madras Medical college and Rajiv Gandhi Government Hospital, Chennai- 3

Name and address of the sponsor / agency

Nil

Documentation of the informed consent

I have read the information in this form (or it has been read to me). I was free to ask any questions and they have been answered. I am over 18 years of age and, exercising my free power of choice, hereby give my consent to be included as a participant in "**ANALYSIS OF COLLAGEN STATUS IN WOMEN WITH GENUINE STRESS INCONTINENCE**"

1) I have read and understood this consent form and the information provided to me.

2) I have had the consent document explained to me.

3) I have been explained about the nature of the study.

4) I have been explained about my rights and responsibilities by the investigator.

5) I have been informed the investigator of all the treatments I am taking or have taken in the past ____ months including any native (alternative) treatment.

6) I have been advised about the risks associated with my participation in this study.

7) I agree to cooperate with the investigator and I will inform him/her immediately if I suffer unusual symptoms.

8) I have not participated in any research study within the past _____month(s).

9) I am aware of the fact that I can opt out of the study at any time without having to give any reason and this will not affect my future treatment in this hospital.

10) I am also aware that the investigator may terminate my participation in the study at any time, for any reason, without my consent.

11) I hereby give permission to the investigators to release the information obtained from me as result of participation in this study to the sponsors, regulatory authorities, Govt. agencies, and IEC. I understand that they are publicly presented.

12) I have understood that my identity will be kept confidential if my data are publicly presented

13) I have had my questions answered to my satisfaction.

14) I have decided to be in the research study.

I am aware that if I have any question during this study, I should contact the investigator. By signing this consent form I attest that the information given in this document has been clearly explained to me and understood by me, I will be given a copy of this consent document.

For adult participants:

Name and signature / thumb impression of the participant (or legal representative if participant incompetent)

Name _____
Signature_____ Date_____

Name and Signature of impartial witness (required for illiterate patients):

Name _____
Signature_____ Date_____

Address and contact number of the impartial witness:

Name and Signature of the investigator or his representative obtaining consent:

Name _____
Signature_____ Date_____

For Children being enrolled in research:

Whether child's assent was asked: Yes / No (Tick one)

[If the answer to be above question is yes, write the following phrase:

You agree with the manner in which assent was asked for from your child and given by your child. You agree to have your child take part in this study].

[If answer to be above question No, give reason (s) :_____.

Although your child did not or could not give his or her assent, you agree to your child's participation in this study.

Name and Signature of / thumb impression of the participant's parent(s) (or legal representative)

Name _____
Signature_____ Date_____

Name _____
Signature_____ Date_____

Name and Signature of impartial witness (required for parents of participant child illiterate):

Name _____
Signature_____ Date_____

Address and contact number of the impartial witness: _____

Name and Signature of the investigator or his representative obtaining consent :

Name _____
Signature_____ Date_____

NOTE

ஆராய்ச்சி ஒப்புதல் படிவம்

ஆராய்ச்சி தலைப்பு

சிறுநீர்க்கசிவு உள்ள பெண்களின் இணைப்புத்திசு நிலைக்கும், சிறுநீர்க்கசிவு இல்லாத பெண்களின் இணைப்புத்திசு நிலைக்கும் இடையை உள்ள மாறுதல்கள் பற்றிய ஒப்பிடுதல்

ஆராய்ச்சி நிலையம் : சிறுநீரகவியல் மற்றும் நோய்குறியியல் துறைகள்,
இராஜீவ் காந்தி அரசு பொது மருத்துவமனை,
சென்னை மருத்துவக் கல்லூரி, சென்னை - 03.

பங்கு பெறுவரின் பெயர் :
பாலினம் :
பங்குபெறபவரின் எண் :

பங்கு பெறுபவர் இதனை (✓) குறிக்கவும்

மேலே குறிப்பிட்டுள்ள மருத்துவ ஆய்வின் விவரங்கள் எனக்கு விளக்கப்பட்டது. என்னுடைய சந்தேகங்களை கேட்கவும், அதற்கான தகுந்த விளக்கங்களை பெறவும் வாய்ப்பளிக்கப்பட்டது. ☐

நான் இவ்வாய்வில் தன்னிச்சையாகதான் பங்கேற்கிறேன். எந்த காரணத்தினாலோ எந்த கட்டத்திலும் எந்த சட்ட சிக்கலுக்கும் உட்படாமல் நான் இவ்வாய்வில் இருந்து விலகி கொள்ளலாம் என்றும் அறிந்து கொண்டேன். ☐

இந்த ஆய்வு சம்பந்தமாகவோ, இதை சார்ந்த மேலும் ஆய்வு மேற்கொள்ளும் போதும் இந்த ஆய்வில் பங்குபெறும் மருத்துவர் என்னுடைய மருத்துவ அறிக்கைகளை பார்ப்பதற்கு என் அனுமதி தேவையில்லை என அறிந்து கொள்கிறேன். நான் ஆய்வில் இருந்து விலகிக் கொண்டாலும் இது பொருந்தும் என அறிகிறேன். ☐

இந்த ஆய்வின் மூலம் கிடைக்கும் தகவல்களையும், பரிசோதனை முடிவுகளையும் மற்றும் சிகிச்சை தொடர்பான தகவல்களையும் மருத்துவர் மேற்கொள்ளும் ஆய்வில் பயன்படுத்திக்கொள்ளவும் அதை பிரசுரிக்கவும் என் முழு மனதுடன் சம்மதிக்கின்றேன். ☐

இந்த ஆய்வில் பங்கு கொள்ள ஒப்புக்கொள்கிறேன். எனக்கு கொடுக்கப்பட்ட அறிவுரைகளின்படி நடந்து கொள்வதுடன் 'இந்த ஆய்வை மேற்கொள்ளும் மருத்துவ அணிக்கு உண்மையுடன் இருப்பேன் என்று உறுதியளிகிறேன். எனது உடல் நலம்பாதிக்கப்பட்டாலோ அல்லது எதிர்பாராத வழக்கதிற்கு மாறான நோய்க்குறி தென்பட்டாலோ உடனே அதை மருத்து அணியிடம் தெரிவிப்பேன் என உறுதி அளிக்கிறேன். ☐

பங்கேற்பவரின் கையொப்பம் ..... இடம்..... தேதி.....

கட்டைவிரல் ரேகை

பங்கேற்பவரின் பெயர் மற்றும் விலாசம் .....

ஆய்வாளரின் கையொப்பம் ..... இடம்..... தேதி.....

ஆய்வாளரின் பெயர் .....

APPENDIX-II
PROFORMA

ANALYSIS OF COLLAGEN STATUS IN WOMEN WITH GENUINE STRESS INCONTINENCE

NAME

AGE & SEX

URO NO BODY WEIGHT

ADDRESS

HISTORY

INCONTINENCE STRESS INCONTINENCE

URGE INCONTINENCE

WET AT REST

WET AT POSTURE

CONTINUOUS DRIBBLING

FREQUENCY - DIURNAL /NOCTURNAL

DAILY WATER INTAKE

LOIN PAIN

DYSURIA/ PYURIA

PAST HOSTORY

DM/ HT/ PT

NEUROLOGICAL ILLNESS

OBSTETRIC HISTORY

PREVIOUS SURGERY

GENERAL EXAMINATION

PULSE

BLOOD PRESSURE

PER ABDOMINAL EXAMINATION

PER VAGINAL EXAMINATION

PER RECTAL EXAMINATION

CYSTOSCOPY / BONNEY'S TEST

INVESTIGATIONS

- HAEMOGLOBIN
- BLOOD UREA
- BLOOD SUGAR
- Sr.CREATININE
- Sr.ELECTROLYTES
- USG – KUB
- URO DYNAMIC EVALUATION

IMMUNOHISTOCHEMICAL STANING

CASES

Name	Age	parity	MODE OF DELIVERY	Weight	Dm	HT	Daily water intake in liters	Voids/day	VoidS/night	Cystoscopy	% of type 1 collagen	% of type 111 collagen	collagen 1/111 ratio
BHUVANESHWARY	35	p2L2	LN	50	N	N	3	7	2	N/SL+	30	59	0.5
PERIYANAYAKI	43	P3L3	LN	54	N	N	2	6	0	N/SL+	28	40	0.7
VISALAKSHI	40	P1L1	LSCS	64	N	N	2.5	7	1	N/SL+	40	54	0.74
MALLIGA	37	NULLI	NO	52	N	N	2	5	2	N/SL+	34	44	0.77
KANNALAGI	44	P2L2	LN	60	Y	N	2	6	1	N/SL+	32	42	0.76
KANAGA	32	P1L1	LN	70	N	N	3	7	0	N/SL+	17	54	0.31
INBAVALLI	38	P12L2	LN/LSCS	44	N	N	2	5	1	N/SL+	22	34	0.64
SAVITHRI	48	P3L3	LN	48	Y	Y	3	7	2	N/SL+	10	46	0.21
LALITHA	44	P1L1	LSCSLN	56	N	N	2	6	1	N/SL+	18	42	0.42
SANTHA	36	P2L2	LN	57	N	N	2	6	2	N/SL+	16	36	0.44
MARAGADHAM	45	NULLI	LN	66	Y	N	3	4	1	N/SL+	20	38	0.52
GEETHA	42	P2L2	LN	71	N	N	2	5	0	N/SL+	18	54	0.33
ANJALAXMI	37	P1L1	LSCS	68	N	N	3	6	1	N/SL+	14	36	0.38
SUNDHARI	43	P2L2	LN	70	N	Y	3.5	4	0	N/SL+	12	46	0.26
ANITA	40	P1L1	LSCS	64	N	N	2	6	1	N/SL+	16	44	0.36
SUDHA	44	P2L2	LN/LSCS	48	N	N	3	6	2	N/SL+	22	46	0.47
REGINA	32	P1L1	LSCS	54	N	N	2.5	5	0	N/SL+	24	56	0.42
MANGAI	30	NULLI	NO	60	N	N	2	4	0	N/SL+	20	47	0.42
MARAGADHAM	42	P2L2	LN	64	N	N	3	6	1	N/SL+	18	53	0.33
SARASWATHY	46	P1L1	LN	74	Y	N	3.5	4	0	N/SL+	23	48	0.47
MALATHY	36	P2L2	LN	68	N	N	2	6	0	N/SL+	22	35	0.62
LAXMI	40	P3L3	LN	80	N	Y	2.5	5	1	N/SL+	16	47	0.34
MEENATCHI	44	P1L1	LSCS	49	N	N	2	4	2	N/SL+	19	54	0.35
ANJALI	48	P2L2	LN	57	Y	Y	3.5	6	0	N/SL+	24	60	0.4
MAHESHWARY	38	P2L2	LN/LSCS	63	N	N	2.5	5	1	N/SL+	18	44	0.4

CONTROLS

Name	Age	parity	MODE OF DELIVERY	Weight	Dm	HT	Daily water intake in liters	Voids/day	Voids/night	Cystoscopy	% of type 1 collagen	% of type 111 collagen	collagen 1/111 ratio
DHANALAXMI	44	P2L2	LN	64	N	N	3.5	6	1	N/SL-	56	28	2
LAXMI	36	P2L2	LN/LSCS	74	N	N	3	6	0	N/SL-	44	18	2.44
RANI	42	P1L1	LN	65	N	N	2.5	4	0	N/SL-	52	12	4.33
RADHIGA	45	P2L2	LN	54	N	N	3	5	1	N/SL-	48	16	3
SELVI	35	P2L2	LN/LSCS	52	N	N	2	6	0	N/SL-	44	12	3.66
JEYALAXMI	38	P3L3	LN	49	N	N	3	5	0	N/SL-	38	18	2.11
MEENAL	48	P2L2	LN	50	Y	N	2.5	6	1	N/SL-	44	14	3.14
JOTHY	29	P1L1	LN	64	N	N	3.5	5	2	N/SL-	46	12	3.83
RANI	32	P2L2	LN/LSCS	68	N	N	2	6	1	N/SL-	38	18	2.11
PANDIAMMAL	45	P3L3	LN	70	N	Y	3	7	0	N/SL-	54	22	2.45
GIVINDHAMMAL	47	NULL	NO	78	Y	N	2.5	5	2	N/SL-	44	24	1.83
MUNIAMMAL	38	P2L2	LN	80	N	N	3	6	0	N/SL-	58	19	3.05
ESWARY	31	NULL	NO	68	N	N	2.5	4	0	N/SL-	47	14	3.35
SUJATHA	34	P2L2	LN	72	N	N	2	5	1	N/SL-	38	12	3.16
PARVEEN	44	P2L2	LN/LSCS	75	N	N	2	6	1	N/SL-	54	29	1.86
SARASWATHY	44	P2L2	LN	72	N	N	2.5	6	1	N/SL-	46	32	1.43
PRIYA	36	P1L1	LN	76	N	N	2	7	0	N/SL-	40	30	1.33
HEMAVATHY	28	NULL	NO	64	N	N	3.5	4	2	N/SL-	56	18	3.11
THAMARAI	48	P2L2	LN/LSCS	78	N	Y	3	6	0	N/SL-	44	20	2.2
GOMATHY	36	P3L3	LN	74	N	N	2.5	4	-2	N/SL-	40	18	2.22
PANDEESWARY	42	P2L2	LN/LSCS	74	Y	N	3	5	0	N/SL-	54	12	4.5
THILAGAVATHY	43	P3L3	LN	68	N	N	3.5	6	0	N/SL-	44	18	2.44
PADMA	35	P1L1	LN	64	N	N	3	5	1	N/SL-	40	16	2.5
THAMEEN	42	P2L2	LSCS	60	N	N	3	7	0	N/SL-	56	18	3.11
BANU	44	NULL	NO	58	N	Y	2	5	1	N/SL-	64	14	4.57

INSTITUTIONAL ETHICS COMMITTEE
MADRAS MEDICAL COLLEGE, CHENNAI-3

Telephone No: 04425305301
Fax : 04425363970

CERTIFICATE OF APPROVAL

To
Dr. Hemalatha K
PG in MCh Urology
Madras Medical College, Ch-3

Dear Dr. Hemalatha K

The Institutional Ethics Committee of Madras Medical College reviewed and discussed your application for approval of the proposal entitled "Analysis of collagen status in women with genuine stress incontinence" No. 19022012.

The following members of Ethics Committee were present in the meeting held on 22.02.2012 conducted at Madras Medical College, Chennai-3.

1.	Dr. S.K. Rajan, MD.FRCP.DSc	-- Chairperson
2.	Prof. Pregna. B. Dolia MD Vice Principal, Madras Medical College, Chennai-3	-- Member Secretary
3.	Prof. Md Ali. MD DM Prof & HOD, Dept. of MGE, MMC, Chennai-3	-- Member
4.	Prof Vasanthi MD Prof of Pharmacology, MMC, Ch-3	-- Member
5.	Prof. E. Dhandapani, MD Prof of Internal Medicine, MMC, Ch-3	-- Member
6.	Thiru. S. Govindasamy . BA.BL	-- Lawyer
7.	Tmt. Arnold Soulina MA , MSW	-- Social Scientist

We approve the proposal to be conducted in its presented form

Sd /. Chairman & Other Members

The Institutional Ethics Committee expects to be informed about the progress of the study, any SAE occurring in the course of the study, any changes in the protocol and patient information / informed consent and asks to be provided a copy of the final report

Member Secretary, Ethics Committee

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INTRODUCTION Genuine stress urinary incontinence is defined as a condition in which involuntary urine loss occurs in an intact urethra, when intra vesical pressure exceeds intra urethral pressure without a detrusor contraction during physical exertion. Genuine stress urinary incontinence results from an anatomical defect of the support of the urethra vesical junction and proximal urethra resulting in displacement of these during stress, but the precise mechanism is poorly understood. Intact functional collagen fibres are needed for mechanical stability of the genitourinary tract, which support the bladder neck, urethra and pelvic organs. In a study by Ulmsten et al and Versi et al showed...

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